
BIOGRAPHICAL SKETCH

NAME: Paoletti, Francesca

POSITION TITLE: CNR Researcher (Ricercatore, III livello professionale, Consiglio Nazionale delle Ricerche), Institute of Crystallography

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Trieste, Italy	Master Degree (Laurea)	07/2001	Chemistry
SISSA (International School for Advanced Studies), Trieste, Italy	PhD	04/2006	Structural and Functional Genomics
SISSA (International School for Advanced Studies), Trieste, Italy	Postdoctoral	10/2006	Structural biology / Neurobiology
Fondazione EBRI – Rita Levi- Montalcini, Roma, Italy	Postdoctoral	05/2008	Structural biology / Neurobiology

A. Personal Statement

My main research interest is related to the biophysics and structural biology of the proteins of the neurotrophin family. In particular, the focus of my work is on the structure/function relationships of the neurotrophins, their receptors and other interacting partners. Among these, a set of specific antibodies and their possible applications in neurodegeneration, as well as a set of small endogenous ligand interactors, which might be explored as potential pharmaceutical agents. The main goal is achieved through an integrative experimental and computational structural biology approaches, using complementary techniques: NMR, solution x-ray scattering (SAXS), protein crystallography, surface plasmon resonance (SPR), mass spectrometry and bioinformatics. One central topic has been on the structural study of proNGF, the natural precursor of NGF, the prototype of the neurotrophin family. proNGF was described to have a complementary and somewhat opposite bio-activity in comparison to mature NGF. We have been able to describe the first structural information on this protein, which is characterized by an Intrinsically Unstructured Domain (IUD). Its nature of "hybrid" ordered-partially unstructured protein, makes a challenge its in vitro characterization. Being an highly dynamic and conformational plastic system I tailored my research activities on the structural studies in solution, namely NMR spectroscopy.

As a complement to the structural studies, I perform protein-protein functional interactions investigations, with traditional immunoassays as well as using SPR. These are needed for a comprehensive description of the molecular and structural determinants of the biological role of proNGF, especially in neurodegeneration and in the functional equilibrium between the precursor and the mature form of the protein. Moreover, SPR technique is applied to the development of novel immunoassays with diagnostic/therapeutic interests.

Studies, have been focused on i) a recombinant mutated form of NGF related to the genetic disease HSNV and on its precursor and ii) to proBDNF and its mutant V66M, involved in psychiatric diseases.

Most recently I directed my activities on dynamical aspects of the interactions of neurotrophins and small ligands of potential pharmaceutical interest by using complementary techniques, namely

ligand titration by solution NMR, vibrational spectroscopies, molecular dynamics simulations, as well as protein-protein interaction studies via SPR and Isothermal Titration Calorimetry.

Ongoing and recently concluded projects that I would like to highlight include:

- Instruct-ERIC Pilot R&D project call 2023. Duration: June 2023 – June 2024
Title: “Illuminating the role of the intrinsically disordered regions of pro-neurotrophins and their interacting cartography with ligands and receptors by integrative structural biology with hybrid methods.” **Paoletti (PI)**
- Joint Laboratories Research Project, CNR. Duration: January 2024 – December 2026
Title: “NT-mINT: Structure-based identification of neurotrophins and pro-neurotrophins molecular interactors to target neurodegeneration and pain”. **Paoletti (PI)**

Selected citations:

1. **Paoletti F.**, Covaceuszach S., Konarev P.V., Gonfloni S., Malerba F., Schwarz E., Svergun D.I., Cattaneo A., Lamba D., (2009) Intrinsic structural disorder of mouse proNGF, *Proteins*. 2009 Jun;75(4):990-1009
2. Manca A., Capsoni S., Di Luzio A., Vignone D., Malerba F., **Paoletti F.**, Brandi R., Arisi I., Cattaneo A., Levi-Montalcini R. (2012) Nerve growth factor regulates axial rotation during early stages of chick embryo development. *Proc Natl Acad Sci U S A*. 109:2009-2014.
3. **Paoletti F.**, de Chiara C., Kelly G., Covaceuszach S., Malerba F., Yan R., Lamba D., Cattaneo A., Pastore A., (2016) Conformational rigidity within plasticity promotes differential target recognition of Nerve Growth Factor. *Frontiers in Molecular Biosciences*, Dec 26;3:83
4. **Paoletti F.**, Merzel F., Cassetta A., Ogris I., Covaceuszach S., Grdadolnik J., Lamba D., Golič Grdadolnik S., (2021) Endogenous modulators of neurotrophin signaling: Landscape of the transient ATP-NGF interactions. *Comput Struct Biotechnol J*. May 7;19:2938-2949
5. **Paoletti F.**, ATP binding to Nerve Growth Factor (NGF) and pro-Nerve Growth Factor (proNGF): an endogenous molecular switch modulating neurotrophins activity. *Biochem Soc Trans*. 2024 Jun 26;52(3):1293-1304. doi: 10.1042/BST20231089

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointments

12/2022 - present	Researcher (Ricercatore III livello) Institute of Crystallography, CNR, Trieste Outstation
11/2017- 11/2022	Research Assistant at the National Institute of Chemistry, Ljubljana (Slovenia). Department D01 – Laboratory for molecular structural dynamics, Group of Prof. Dr. Simona Golič Grdadolnik
11/2016 – 06/2017	Teacher (Science, chemistry, biology) at the High School (Liceo Scientifico) “Galileo Galilei”, Trieste, Italy
09/2014 – 08/2016	Research Fellow (Assegno di Ricerca) at Scuola Normale Superiore (Pisa) on the European Project PAINCAGE (UE14PAIN FP7-HEALTH-2013-INNOVATION-1 PAINCAGE “The NGF system and its interplay with endocannabinoid signalling, from peripheral sensory terminals to the brain: new targets for the development of next generation drugs for neuropathic pain”).
05/2008 - 07/2014	Research Fellow at Fondazione EBRI–R.Levi-Montalcini (Roma), Group: Neurotrophic Factors and neurodegenerative Diseases. PI: Prof. A.Cattaneo
06/2011 - 10/2011	Guest scientist at National Institute for Medical Research (NIMR) - London, UK (Research group of Prof. A.Pastore) with a Royal Society - Accademia Nazionale dei Lincei Fellowship
08/2011 – 09/2011	Guest Scientist at the University of Helsinki, Institute of Biotechnology, in the Group of Dr. Hideo Iwai (Biomolecular NMR & Protein Engineering)
12/2009 - 02/2010	Guest scientist at National Institute for Medical Research (NIMR) - London, UK (Research group of Prof. A. Pastore) with EMBO Short term fellowship

- 11/2006 - 05/2008 Post-doctoral fellow at Fondazione EBRI–R.Levi-Montalcini (Roma), Group: Neurotrophic Factors and neurodegenerative Diseases. PI: Prof. A.Cattaneo
- 02/2006 - 10/2006 Post-doctoral fellow at SISSA (Scuola Internazionale Superiore di Studi Avanzati), Trieste – Neurobiology Sector
- 11/2005 - 01/2006 Fellow at SISSA, Trieste – Neurobiology Sector
- 11/2001 - 10/2005 Doctoral fellow at the PhD program in structural and functional genomics at SISSA, Trieste – Neurobiology Sector.
- 10/2004 - 03/2005 Visiting graduate student at the Institute for Protein Biotechnology of the Martin Luther University, Halle-Wittenberg - Halle (Saale), Germany, with EMBO Short term fellowship. Research group: Prof. Dr. Elisabeth Schwarz
- 05/2001 - 09/2001 Undergraduate fellow at SISSA (Trieste) – Biophysics Sector

Awards

- 06/2011 - 10/2011 Royal Society bursary from Accademia Nazionale dei Lincei as visiting Post-doctoral scientist at the NIMR (National Institute for Medical Research) – Division of Molecular Structure, London (UK), Laboratory of Dr. A.Pastore.
- 06/2010 General Travel Grant awarded by the Biochemical Society for the “International Meeting NGF2010. Neurotrophic Factors in Health and Disease”, Helsinki (Finland)
- 12/2009 - 02/2010 EMBO Short Term Fellowship as visiting Post-doctoral scientist at the NIMR (National Institute for Medical Research) – Division of Molecular Structure, London (UK), in the Laboratory of Dr. A.Pastore. Ref.: ASTF 70.00-2009.
- 10/2004 - 12/2004 EMBO Short Term Fellowship as visiting PhD student at the Institute of Biotechnology, Halle (Saale) Germany, in the group of prof. Rainer Rudolph and Dr. Elisabeth Schwarz. Reference: ASTF 225-2004.
- 06/2002 Selected for and granted a fellowship from Cold Spring Harbor Laboratory (New York) for the practical course “Molecular Cloning of Neural Genes”.

C. Contributions to Science

- My undergraduate training in chemistry was complemented with a selection of molecular biology and biochemistry courses, to strengthen a global biochemistry curriculum. The lab work for to the Laurea degree was at the edge between organic chemistry and biology, I worked on the development of a protocol for the synthesis, purification and characterization of a novel fluorescent dye for the identification of local protein synthesis in vivo into the cell.
 - Paoletti F.**, Ainger K., Donati I., Scardigli R., Vetere A., Cattaneo A., Campa C. (2010) Novel fluorescent cycloheximide derivatives for the imaging of protein synthesis, *Biochem Biophys Res Commun.*, 396(2):258-64.
- The PhD project (2001-2005) contributed to the investigation of the structure and function of mouse proNGF, with the aim of getting new insights into its molecular function. The experimental difficulties in the recombinant expression of the protein and of its nature as intrinsically unstructured protein made the project quite challenging. Nevertheless, I was able to obtain the first structural information on a pro-neurotrophin, namely proNGF by Small Angle X-ray Scattering. During the PhD, a specific antibody against proNGF was selected in the form of scFv using the SPLINT technology.
 - Paoletti F.**, Covaceuszach S., Schwarz E., Stubbs M., Rudolph R., Cattaneo A. and Lamba D. (2005) Towards a comprehension of the structure of mouse proNGF. *Acta Cryst. A61, C234-C235* (Abstracts of the XX IUCr Congress)
 - Paoletti F.** Konarev P.V., Covaceuszach S., Schwarz E., Cattaneo A., Lamba D., Svergun D.I. (2006) Structural and functional properties of mouse proNGF. *Biochem Soc Trans.* 2006 Aug;34(Pt4):605-6.
 - International patent query: Title: Compositions able to prevent neurodegenerative processes and methods of assaying the same. Patent n.: WO2005044293. Publication date: 2005-05-19. Patent inventors: Capsoni S., Cattaneo A., Covaceuszach S., **Paoletti F.**, Visintin M.

3. During the first post-doctoral fellowship at EBRI (2006-2008), I consolidated the project on the tridimensional structure of proNGF. Moreover, I started gaining experience in complementary fields, linked to the biology of neurotrophins and on their clinical applications. Moreover, I was directly involved in the setting-up of the facilities for the expression and purification of recombinant proteins at EBRI, as well as their biochemical characterization. I was involved in many of the multi-disciplinary projects of the Institution based on NGF, as well as on Grant proposal writing and submission, and subsequent reporting.
 - a. **Paoletti F.**, Covaceuszach S., Konarev P.V., Visintin M., Schwarz E., Svergun D.I., Lamba D., Cattaneo A., (2007) Structural basis for the selectivity of recombinant mouse short proNGF versus NGF towards its binding partners. *Ann.Meet.Soc. for Neuroscience*
 - b. **Paoletti F.**, Covaceuszach S., Konarev P.V., Gonfloni S., Malerba F., Schwarz E., Svergun D.I., Cattaneo A., Lamba D., (2009) Intrinsic structural disorder of mouse proNGF, *Proteins*. 75(4):990-1009.
 - c. Cattaneo A., Capsoni S., **Paoletti F.**, (2008) Towards Non Invasive Nerve Growth Factor Therapies for Alzheimer's Disease. *Journal of Alzheimer's Disease*, 15, 255-283.
 - d. Cattaneo A., Capsoni S., **Paoletti F.**, (2009) A New Generation of Noninvasive NGF-Based Therapies for Alzheimer's Disease, in *RSC Drug Discovery Series No. 3*, RSC Publishing, Chapter 17, 43-77
4. During the fellowship at EBRI (2008-2014) the involvement in the scientific projects was multidisciplinary as detailed below. I was also directly involved in Grant proposal writing (from Italian, European and international agencies) and submission, and subsequent reporting.
 - a. the biochemical characterization of the NGF/proNGF system in a new mouse model
 - i. Tiveron C., Fasulo L., Capsoni S., Malerba F., Marinelli S., **Paoletti F.**, Piccinin S., Scardigli R., Amato G., Brandi R., Capelli P., D'Aguzzo S., Florenzano F., La Regina F., Lecci A., Manca A., Meli G., Pistillo L., Berretta N., Nisticò R., Pavone F., Cattaneo A. (2013). ProNGF/NGF imbalance triggers learning and memory deficits, neurodegeneration and spontaneous epileptic-like discharges in transgenic mice. *Cell Death and Differentiation* 20, 1017-1030.
 - b. the investigation on the cellular effects of NGF vs proNGF in PC12 cells as a model system, through a functional genomic approach
 - i. D'Onofrio M.[#], **Paoletti F.**[#], Arisi I., Brandi R., Malerba F., Fasulo L., Cattaneo A. (2011) NGF and proNGF Regulate Functionally Distinct mRNAs in PC12 Cells: An Early Gene Expression Profiling. *PLoS ONE* 6(6): e20839. [#] **Equal contribution**
 - ii. Arisi I., D'Onofrio M., Brandi R., Malerba F., **Paoletti F.**, Storti A.E., Florenzano F., Fasulo L., Cattaneo A. (2014) proNGF/NGF mixtures induce gene expression changes in PC12 cells that neither singly produces. *BMC Neurosci.* 15:48.
 - c. the validation of a specific anti-proNGF antibody as a tool for new specific immunoassays for diagnostic purposes
 - i. **Paoletti F.**, Malerba F., Konarev P.V., Visintin M., Scardigli R., Fasulo L., Lamba D., Svergun D.I., Cattaneo A. (2012) Direct intracellular selection and biochemical characterization of a recombinant anti-proNGF single chain antibody fragment. *Arch Biochem Biophys.* 522:26-36.
 - d. painless NGF as a new clinical application of NGF
 - i. Covaceuszach S., Capsoni S., Marinelli S., Pavone F., Ceci M., Ugolini G., Vignone D., Amato G., **Paoletti F.**, Lamba D., Cattaneo A. (2010) In vitro receptor binding properties of a "painless" ngf mutein, linked to hereditary sensory autonomic neuropathy type v., *Biochem Biophys Res Commun.* 2010 Jan 1;391(1):824-9.
 - ii. Malerba F., **Paoletti F.**, Capsoni S., Cattaneo A. (2011) Intranasal delivery of therapeutic proteins for neurological diseases. *Expert Opin Drug Deliv.* 8(10):1277-96.
 - iii. Capsoni S., Marinelli S., Ceci M., Vignone D., Amato G., Malerba F., **Paoletti F.**, Meli G., Viegi A., Pavone F., Cattaneo A. (2012) Intranasal "painless" Human Nerve Growth Factors Slows Amyloid Neurodegeneration and Prevents Memory Deficits in App X PS1 Mice. *PLoS One.* 7(5):e37555.
 - e. the biophysical and structural analysis of NGF and proNGF via solution NMR and SAXS
 - i. **Paoletti F.**, Malerba F., Kelly G., Noinville S., Lamba D., Cattaneo A., Pastore A. (2011) Conformational plasticity of proNGF. *PLoS ONE.* 6(7) July 2011, e22615
 - ii. Covaceuszach S., Konarev P.V., Cassetta A., **Paoletti F.**, Svergun D.I., Lamba D., Cattaneo A. (2015) The conundrum of the high-affinity NGF binding site formation unveiled? *Biophys J.* 2015 Feb 3;108:687-697.

- f. new biological aspects of NGF, as a focus of EBRI work in Rita Levi-Montalcini group
 - i. Manca A., Capsoni S., Di Luzio A., Vignone D., Malerba F., **Paoletti F.**, Brandi R., Arisi I., Cattaneo A., Levi-Montalcini R. (2012) Nerve growth factor regulates axial rotation during early stages of chick embryo development. *PNAS*;109:2009-2014.
5. During the “Assegno di Ricerca” at SNS (2014-2016) in the context of the EU-funded project “PAINCAGE” (2014-2017) the experimental activity was mainly focused on:
 - a. the biochemistry and structural biology of painless NGF and proNGF, with attention at the possible clinical applications
 - i. Paoletti F., Malerba F., Bruni Ercole B., Lamba D., Cattaneo A. (2015) A comparative analysis of the structural, functional and biological differences between Mouse and Human Nerve Growth Factor. *Biochim Biophys Acta*. 1854:187-197.
 - ii. Malerba F., **Paoletti F.**, Bruni Ercole B., Materazzi S., Nassini R., Coppi E., Patacchini R., Capsoni S., Lamba D., Cattaneo A. (2015) Functional Characterization of Human ProNGF and NGF Mutants: Identification of NGF P61SR100E as a "Painless" Lead Investigational Candidate for Therapeutic Applications. *PLoS One*. 10(9):e0136425.
 - b. the elucidation of the first NGF NMR solution structure and its dynamical properties
 - i. **Paoletti F.**, de Chiara C., Kelly G., Covaceuszach S., Malerba F., Yan R., Lamba D., Cattaneo A., Pastore A., (2016) Conformational rigidity within plasticity promotes differential target recognition of Nerve Growth Factor. *Front. in Mol. Biosciences*, 3:83.
 - c. the investigation of the properties of the proNGF pro-peptide
 - i. Yan R., Yalinca H., **Paoletti F.**, Gobbo F., Marchetti L., Kuzmanic A., Lamba D., Gervasio F.L., Konarev P.V., Cattaneo A., Pastore A., (2019) The Structure of the Pro-domain of Mouse proNGF in Contact with the NGF Domain. *Structure*. 27(1):78-89.e3
 - d. the investigation of new immunoassays for the selective detection of NGF and proNGF
 - i. Malerba F.[#], **Paoletti F.**[#], Cattaneo A. (2016) NGF and proNGF reciprocal interference in immunoassays: open questions, criticalities and ways forward. *Frontiers in Molecular Neuroscience*, 2016 Aug 3;9:63. **# Equal contribution**
6. During the research position at the National Institute of Chemistry (Slovenia) (2017-2022) the experimental interest was moved to aspects related to the interaction of proteins and small molecules, aimed at the investigation on possible pharmaceutical applications. In particular, the work has been focused on a project regarding the interaction of NGF with ATP and other small ligands of pharmaceutical interest, using solution NMR coupled with other biophysical and computational techniques.
 - a. **Paoletti F.**, Merzel F., Cassetta A., Ogris I., Covaceuszach S., Grdadolnik J., Lamba D., Golič Grdadolnik S., Endogenous modulators of neurotrophin signaling: Landscape of the transient ATP-NGF interactions. *Comput Struct Biotechnol J*. (2021) 19:2938-2949.
 - b. **Paoletti F.** and Lamba D., Small Endogenous Ligands Modulation of Nerve Growth Factor Bioactivity: A Structural Biology Overview. *Cells*, (2021), 10, 3462
 - c. **Paoletti F.**^{*}, Covaceuszach S.^{*}, Cassetta A., Calabrese A. N., Novak U., Konarev P., Grdadolnik J., Lamba D., Golič Grdadolnik S., Distinct conformational changes occur within the intrinsically unstructured pro-domain of pro-Nerve Growth Factor in the presence of ATP and Mg²⁺. *Protein Science*, 2023, 32(2):e4563. ***Equal contribution**
7. Since 01/12/2022 I was appointed as a Researcher at the Institute of Crystallography of CNR (National Research Council of Italy), with an independent position. I have been awarded with two research grants, reported in section A. I have been starting working on the description of the molecular determinants of the interaction between neurotrophins and endogenous ligands, through an integrative structural biology approach. I have also been involved in the establishment of a scientific agreement between the IC-CNR and the NIC (Ljubljana, Slovenia).
 - a. **Paoletti F.**, Goričan T, Cassetta A, Grdadolnik J, Toporash M, Lamba D, Golič Grdadolnik S, Covaceuszach S., Unveiling the Solvent Effect: DMSO Interaction with Human Nerve Growth Factor and Its Implications for Drug Discovery, *Molecules*. 2025 Jul 19;30(14):3030. doi: 10.3390/molecules30143030.
 - b. **Paoletti F.**, ATP binding to Nerve Growth Factor (NGF) and pro-Nerve Growth Factor (proNGF): an endogenous molecular switch modulating neurotrophins activity. *Biochem Soc Trans*. 2024 Jun 26;52(3):1293-1304. doi: 10.1042/BST20231089.